

1983). A useful system for stable high level expression of mammalian cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). A useful high expression vector, PMLSV N1/N4, described by Cosman et al., *Nature* 312:768, 1984, has been deposited as ATCC 39890. Additional useful mammalian expression vectors are described in EP-A-0367566, and in U.S. Patent Application Serial No. 07/701,415, filed May 16, 1991, incorporated by reference herein. The vectors can be derived from retroviruses. In place of the native signal sequence, a heterologous signal sequence can be added, such as the signal sequence for IL-7 described in United States Patent 4,965,195; the signal sequence for IL-2 receptor described in Cosman et al., *Nature* 312:768 (1984); the IL-4 signal peptide described in EP 367,566; the type I IL-1 receptor signal peptide described in U.S. Patent 4,968,607; and the type H IL-1 receptor signal peptide described in EP 460,846.

[097] The polypeptides of the invention and the nucleic acid molecules encoding them can also be used as reagents to identify (a) proteins that the disclosed polypeptides or their constituent proteins regulate, and (b) other proteins with which it might interact. The disclosed polypeptides can be coupled to a recombinant protein, to an affinity matrix, or by using them as a bait in the yeast two-hybrid system. The use of the yeast two-hybrid system developed by Stanley Fields and coworkers is well known in the art and described in Golemis, E., et al Section 20.1 in: *Current Protocols in Molecular Biology*, ed. Ausubel, F.M., et al., John Wiley & Sons, NY, 1997 and in *The Yeast Two-Hybrid System.*, ed. P.L. Bartel and S. Fields, Oxford University Press, 1997.

LAW OFFICES

FINNEGAN, HENDERSON,  
FARABOW, GARRETT,  
& DUNNER, L.L.P.  
1300 I STREET, N. W.  
WASHINGTON, DC 20005  
202-408-4000

## Antibodies and Peptide Binding Proteins

[098] Purified polypeptides of the invention can be used to generate antibodies that bind to one or more epitopes of the disclosed polypeptide. Such anti-polypeptide antibodies includes polyclonal antibodies, monoclonal antibodies, fragments thereof such as F(ab')<sub>2</sub>, and Fab fragments, as well as any recombinantly produced binding partners. Antibodies are defined to be specifically binding if they bind pine tree polypeptides with a  $K_a$  of greater than or equal to about  $10^7 \text{ M}^{-1}$ . Affinities of binding partners or antibodies can be readily determined using conventional techniques, for example, those described by Scatchard et al., *Ann. N.Y. Acad. Sci.*, 51:660 (1949).

[099] Polyclonal antibodies can be readily generated from a variety of sources, for example, horses, cows, goats, sheep, dogs, chickens, rabbits, mice, hamsters, guinea pigs, or rats, using procedures that are well-known in the art, for example, as described for example, in U.S. Patent 5,585,100, incorporated by reference herein. In general, a composition comprising at least one of the polypeptides of the invention is administered to the host animal, typically through intra-peritoneal or subcutaneous injection. In the case where a peptide is used as the immunogen, it is preferable to conjugated it to a suitable carrier molecule, such as a T-dependent antigen (Bovine Serum Albumin, cholera toxin, and the like). The immunogenicity of the disclosed polypeptides can also be enhanced through the use of an adjuvant, for example, Freund's complete or incomplete adjuvant or alum. Following booster immunizations, small samples of serum are collected and tested for reactivity to the disclosed polypeptides or their constituent epitopes. Examples of various assays useful for such determination include those described in: *Antibodies: A Laboratory Manual*, Harlow and

LAW OFFICES

FINNEGAN, HENDERSON,  
FARABOW, GARRETT,  
& DUNNER, L.L.P.  
1300 I STREET, N. W.  
WASHINGTON, DC 20005  
202-408-4000

Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; as well as procedures such as countercurrent immuno-electrophoresis (CIEP), radioimmunoassay, radio-immunoprecipitation, enzyme-linked immuno-sorbent assays (ELISA), dot blot assays, and sandwich assays, see U.S. Patent Nos. 4,376,110 and 4,486,530, each of which is incorporated by reference in their entirety.

[0100] Monoclonal antibodies (or fragments thereof), directed against epitopes of the disclosed polypeptides can also be readily prepared using well-known procedures, such as, for example, the procedures described in U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993; *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, Kennett, McKearn, and Bechtol (eds.), 1980, each of which is incorporated by reference. Briefly, the host animals, such as mice, are injected intraperitoneally at least once, and preferably at least twice at about 3 week intervals with isolated and purified polypeptides optionally in the presence of adjuvant. Again, if peptide fragments are used they may need to be conjugated to a suitable carrier protein. Mouse sera are then assayed by conventional dot blot technique or antibody capture (ABC) to determine which animal is best to fuse.

Approximately two to three weeks later, the mice are given an intravenous boost of pine tree polypeptides. Mice are later sacrificed and spleen cells fused with commercially available myeloma cells, such as Ag8.653 (ATCC), following established protocols.

Briefly, the myeloma cells are washed several times in media and fused to mouse spleen cells at a ratio of about three spleen cells to one myeloma cell. The fusing agent can be any suitable agent used in the art, for example, polyethylene glycol (PEG).

Fusion is plated out into plates containing media that allows for the selective growth of

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FARABOW, GARRETT,  
& DUNNER, L.L.P.  
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WASHINGTON, DC 20005  
202-408-4000